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Firing pattern of fasciculations in ALS

Evidence for axonal and neuronal origin

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ABSTRACT

Background: In amyotrophic lateral sclerosis (ALS), the origin of fasciculations is disputed. We hypothesized that the discharge pattern of fasciculation potentials (FPs) would be different for FPs arising in the motor axon or in the spinal motor neuron.

Method: FPs were recorded by high-density surface EMG of the biceps brachii or vastus lateralis muscle for 15 minutes in 10 patients with ALS. Records were decomposed into different FP waveforms and their firing moments. Interspike interval (ISI) histograms were constructed for FPs that fired more than 100 times.

Results: Two types of ISI histograms were found. 1) In 23 of 30 different FPs with a total of 8,597 ISIs, the refractory period was 3 to 4 msec. ISIs longer than 15 msec had a Poisson distribution. Five of these 23 FPs discharged doublets with an ISI of approximately 5 msec, indicative of supernormality. This is consistent with the FPs arising in motor axons. 2) In the other 7 FPs, accounting for 11,266 ISIs, the refractory period was 17 to 46 msec. The preferred ISI duration was around 80 msec. Both timing factors are consistent with origin in the spinal motor neuron.

Conclusions: Firing pattern analysis, based on high-density surface EMG, can detect fasciculation potentials (FPs) of axonal and neuronal origin in amyotrophic lateral sclerosis. The two FP types coexist within the same muscle. The recognition that clinically identical fasciculations conceal the existence of two types of FP that can be studied in a noninvasive manner will introduce a new aspect in the research of motor neuron disease. *Neurology*® 2008;70:353-359

GLOSSARY

ALS = amyotrophic lateral sclerosis; **FP** = fasciculation potential; **ISI** = interspike interval; **MU** = motor unit.

Fasciculations, a clinical hallmark of ALS, are defined as random, spontaneous twitchings of a group of muscle fibers belonging to a single motor unit.¹ Superficial fasciculations are visible through the skin; deeper ones can be detected by palpation, ultrasound imaging, or surface EMG.^{2,3} Joint movement of fingers or toes can occur if motor units are enlarged as a result of reinnervation. Fasciculations are often described as irregular with respect to both their firing pattern and the site of visible muscle activation. After the introduction of concentric needle EMG, fasciculation potentials (FPs) were described as abnormal motor unit (MU) discharges and differentiated them from fibrillation potentials.⁴ An FP is defined as the electrical activity associated with a fasciculation and has the configuration of a MU action potential but occurs spontaneously.¹ This means that an FP is distinguished from a voluntarily recruited MU potential solely by its firing pattern. During voluntary movement, MUs discharge in trains. The lowest possible firing rate is usually between 5 and 8/second, but can differ between muscles. FPs can be detected in many diseases of the motor neuron or the peripheral nerve, but also in healthy subjects.^{3,5} This is why fasciculations play only a minor role in the diagnostic criteria for amyotrophic lateral sclerosis (ALS).⁶ Nevertheless, widespread fasciculations prompt a high index

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of suspicion, especially because recently FPs were documented as the only finding in early ALS.⁷

Fasciculations have been shown to arise distally or proximally in the motor axon. This was demonstrated by the observation that FPs were still present immediately after cutting the nerve, and by collision and F-wave experiments.⁸⁻¹⁰

Already in their original description of FPs, Denny-Brown and Pennybacker⁴ proposed that fasciculations originated within the motor neuron itself, because the sporadic discharge of an FP changed into the regular pattern of MU activity. A central origin of FPs in ALS is supported by more recent findings. At least some FPs can be recruited voluntarily and by transcranial magnetic stimulation.^{11,12} In particular in early ALS, fasciculations are related to cortical hyperexcitability.¹³ Another observation suggestive of a spinal or supraspinal origin is the synchrony between FP discharges.¹⁴ Although no cross-correlation analysis has been performed, FPs with different morphology were claimed to discharge simultaneously more often than expected in ALS, but not in Kennedy disease.¹⁵ Short-term synchrony between MUs requires a common synaptic input, e.g., from the corticospinal tract.¹⁶

Taken together, we hypothesized that two distinct populations of fasciculations (and hence of FPs) exist in ALS, namely, peripheral fasciculations arising from membrane instability in motor axons and central fasciculations arising in low-threshold motor neurons that are accidentally recruited by a hyperexcitable corticospinal system or that are hyperexcitable themselves. Because the membrane properties at these different sites are dissimilar, we suggest that the discharge patterns differ between the two populations of FPs.

From the discharge moments of a particular MU or FP, the distribution of interspike intervals (ISIs) can be determined. The ISI distribution is related to the membrane potential characteristics underlying the generation of the spikes.¹⁷ This means

that the dynamics of the membrane currents that generate the discharges can be deduced.¹⁸ The probability of an action potential discharge depends on how close the membrane potential is to the threshold potential. FPs generated by axons can have short ISIs because the axonal membrane recovers its excitability within a few milliseconds, whereas FPs generated by spinal motor neurons must have longer minimal ISIs, because motor neurons become hyperpolarized after discharging an action potential.¹⁹ Therefore, ISI histograms reflect the time course of the membrane potential at the site of generation.

To count ISIs, long-duration recordings and accurate discrimination of FP waveforms are required. Surface EMG has been used for recordings of up to 20 minutes.^{5,20} For technical reasons, analysis was restricted to the overall occurrence of FPs. Accurate discrimination of FPs is possible with needle electrodes, but it is difficult to obtain long and stable recordings of a sufficient number of FPs. We decided to use high-density surface EMG^{21,22} to record FPs in patients with ALS. The multielectrode grid provides a spatial resolution sufficient to differentiate FPs arising in the same muscle but from different MUs.²³ Using a decomposition method²⁴ described earlier, we monitored the spontaneous firing of FPs for 15 minutes.

METHODS Patients. Subjects referred to our outpatient department for evaluation of suspected motor neuron disease were included if fasciculations in either a biceps brachii muscle or a quadriceps muscle were visible. Only data from patients with a sufficient number of FPs (see below) and with ALS confirmed on follow-up were included in this analysis. Ten patients aged 40 to 84 years (mean 62 years) with symptoms attributable to motor neuron disease for 6 to 60 months (mean 31 months) were included. At the time of the surface EMG recording, patients fulfilled the revised El Escorial criteria⁶ for probable (3), probable laboratory supported (4), or definite (3) ALS. Patients gave informed consent to participate in the study. The protocol was approved by the local ethics committee.

High-density surface EMG recording. The high-density surface EMG recording system described earlier was used to record muscle activity. Electrodes were arranged in a 10 × 13 rectangular matrix with an interelectrode distance of 5 mm.²¹ The grid was placed over the belly of the biceps brachii muscle (seven patients) or the lateral vastus muscle

(three patients), parallel to the muscle fibers. The monopolar EMG signals, each referenced to the olecranon or to the patella, were amplified, band-pass filtered (3 to 400 Hz), analog–digital converted with a resolution 0.5 $\mu\text{V/bit}$ at a rate of 2,000 samples/second/channel, and stored on the hard disk of a personal computer for off-line processing.

During EMG recording, the subject was sitting with the arm or leg supported. He or she was asked to relax completely, and visual feedback on the EMG (bipolar derivation, 0.1 mV/div, 100 msec/div) was given to aid relaxation. Short periods with voluntary contractions were identified visually and were excluded from analysis. The duration of data acquisition was set to 15 minutes, which was a compromise between a sufficient number of discharge events, patient comfort, and processing time.

EMG decomposition. To identify FPs, the EMG signal had to be decomposed into the underlying action potential waveforms and their discharge times. A reliable algorithm for MU decomposition^{24,25} was adapted for long-duration recordings and for discrimination of different FPs.²³ In short, the signal was spatially filtered by making a bipolar montage and high-pass filtered at 15 Hz to remove movement artifacts. After action potentials were clustered semiautomatically according to their spatial properties, templates were constructed and used for template matching. The result of automatic full decomposition was checked visually for all firing events. Trains of voluntarily recruited low-threshold MU action potentials are easily and reliably²⁵ identified, and were removed. Superposition of several FPs was resolved by the use spatial information from multiple channels in the same way as in MU potentials. The overall discharge rate of FPs is low, so that the superpositions were less complex than, e.g., in some voluntary contractions or in neuromyotonia.²⁶ Thus, the decomposition procedure enables identification of the firing patterns of the different simultaneously active action potential waveforms, defined as the points in time when they occurred.

Firing pattern analysis. From the firing times of each FP, the ISI duration was calculated as the time (in milliseconds) between two subsequent discharges. ISI histograms were compiled with a bin width of 5 msec. Only FPs with more than 100 discharges were accepted for further analysis. To check for serial correlation between firings, joint ISI plots were generated by plotting the ISI duration as a function of the previous ISI.²⁷

The probability to discharge reflects membrane excitability, i.e., distance to threshold. The instantaneous probability of a spike, as a function of time since the preceding spike, can be expressed as ISI death rate plot. This is also known as death rate function, hazard function, or conditional probability.^{28,29} In contrast to simple descriptive ISI statistics that cannot be interpreted in terms of underlying physiology, the death rate plot reflects the time course of the membrane potential after a spike.^{18,19} Commonly, the death rate is defined as the chance of a discharge within the next time interval (usually 5 msec) of the ISI histogram. To make optimal use of the timing information, we selected the width such that 20 ISIs fell into each bin.¹⁷ This allows a higher time resolution for the more frequently occurring short ISIs. For longer intervals, the time resolution decreases, but noise caused by a low number of counts/bin is avoided. The death rate plot as an estimate for the recovery curve of the membrane potential has been applied to ISIs from regularly dis-

charging motor neurons.³⁰ To interpret the death rate in terms of distance to threshold, the membrane must be in a steady state with a constant threshold and a stable noise level.¹⁸ It is impossible to verify both assumptions for the membrane that generates the fasciculations, but we assume that even in ALS, the spinal motor neuron does not violate them. We also extended the method to the myelinated axon, where relevant membrane noise has been demonstrated.^{26,31}

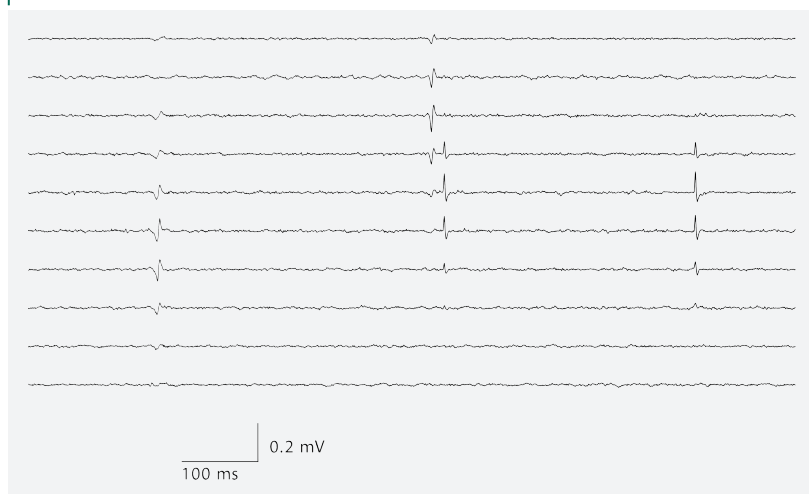
Firing pattern analysis based on decomposition of FPs is a new technique. Reproducibility studies in individual muscles of a single patient have not been performed, because they are not required for the pathophysiologic question whether two populations of firing patterns do exist. However, to establish whether different FPs change their firing pattern over time or to establish this method as a diagnostic tool, repeated recordings should be performed.

RESULTS As an example, a small recording segment is shown in figure 1. Four FPs can be distinguished. Two of them have the same spatial distribution, which means that the waveform and amplitude are the same in all bipolar channels. The two FPs originate from the same MU, and an ISI was calculated.

The ISI distribution of an FP with 1,673 discharges is given in figure 2, A–C. The ISI histogram was constructed with a bin width of 5 msec. To evaluate the first peak in more detail, the ISI histogram with a bin width of 0.5 msec is shown also as inset in figure 2B. The shortest ISI was 3 msec, indicating that the membrane generating the fasciculation then had recovered from its refractory period. The refractory period was followed by a steep increase in firing probability at 4 to 5 msec. The number of longer ISIs gradually decreased for all intervals above 10 msec. In the joint ISI plot (figure 2C), no serial correlation was found. The pattern of fewer discharges with increasing distance from the origin mirrors the ISI histogram. This pattern is compatible with a Poisson process.³² The ISI that followed a very short ISI (<10 msec) was also not different from the preceding ISI. The lower part of the figure (figure 2, D–F) shows another FP with 444 discharges during the same recording. Here, the refractory period was 25 msec, which was followed by high firing probability with a peak at 70 msec (figure 2, D and E). For ISIs longer than 200 msec, the histogram indicated a constant firing probability. The FP fired sometimes in short bursts with ISIs of approximately 70 msec, but any combination of ISIs was possible (figure 2F).

Diagrams as shown in figure 2 were generated for all FPs separately. Two completely distinct discharge patterns were identified. In 7 of 30 FPs (23%), the refractory period was 25 msec or longer and was always accompanied by an in-

Figure 1 Example of a high-density surface EMG recording

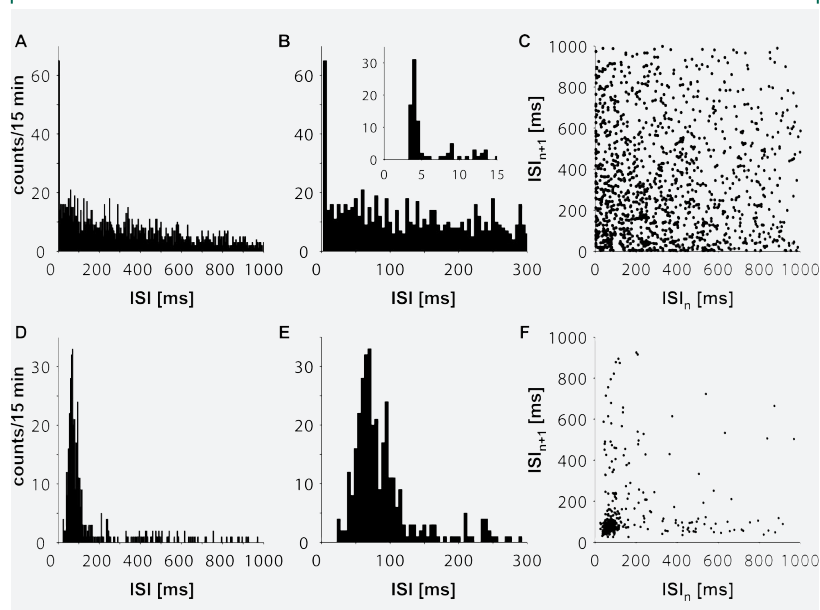


Each channel gives the bipolar signal from two adjacent (in proximal-distal direction) electrodes with 5 mm interelectrode distance. The 10 different channels are separated by 5 mm in the lateral-to-medial direction of the muscle. Four fasciculation potentials (FPs) with three different spatiotemporal distributions were recorded in 1 s. The last two FPs have an identical distribution.

creased discharge probability at approximately 100 msec (figure 2E). In 23 cases (77%), FPs had a short refractory period. Five of these units expressed an increased discharge probability at 5 msec (as in figure 2B), whereas the other 18 units had a flat ISI histogram.

After the FPs were classified according to their

Figure 2 Firing patterns of two different fasciculation potentials (A-C and D-F) that were simultaneously active in the same recording



The interspike interval (ISI) histogram is plotted at two different resolutions (A, D and B, E). The upper (axonal type) fasciculation potential (FP) shows increased firing probability around 4.5 msec (inset in B). The lower (spinal motor neuron type) FP had a longer refractory period (25 msec) and maximum firing probability around 70 msec. Note the absence of serial correlation in the joint ISI plot (C, F) for both FPs.

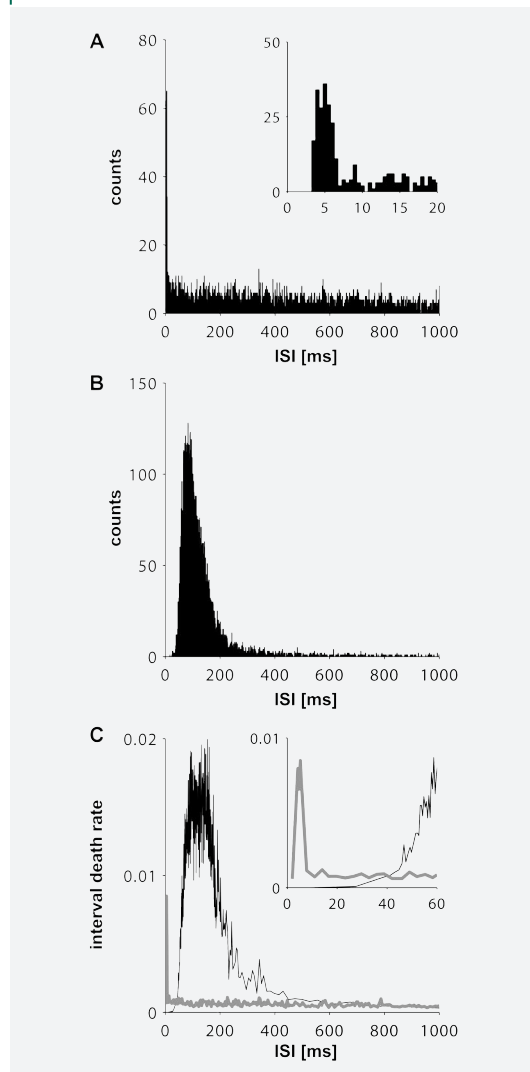
refractory period into the two different patterns, the ISIs of all FPs in each group were pooled. The resulting distribution of 8,597 ISIs from the 23 FPs in the first group is shown in figure 3A. Although only 5 of these 23 FPs had an increased probability of short ISIs, a peak at 5 msec is clearly visible. The ISI histogram (11,266 ISIs) of the 7 remaining FPs showed a peak at 80 to 85 msec (figure 3B). The shortest ISI was 17.5 msec, and 99% of the ISI were 46 msec or longer.

The interval death rate gives the probability that the current ISI will “die” within the next time bin. Because the death of an ISI is defined by discharge of the next spike, this is equivalent to the instantaneous discharge probability. In the first group of FPs (figure 3C, gray line), the chance of firing was independent of the previous FP, except for the first 10 msec of the ISI. This confirms a truly random Poisson process. In the second group (figure 3C, black), the discharge probability was constant, i.e., independent of history from 500 msec onward. For the shorter ISIs, the shape of the interval death rate function was not essentially different from that of the ISI histogram.¹⁹

DISCUSSION We investigated the firing pattern of FPs in patients with ALS, using high-density surface EMG. On the basis of firing pattern information alone, we were able to distinguish two distinct types of fasciculations. Both types of fasciculations could be present in the same muscle. The result confirms the hypothesis that fasciculations can be generated by the axonal membrane and also by the spinal motor neuron itself.

The ISI histograms were of distinct types, implicating that the FPs of a particular MU are driven either from the axon or from the soma. If the fasciculation would arise at random in the axonal or neuronal membrane, a mixture of the histograms would have been encountered. Obviously, antidromic action potentials can occlude some neuronal discharges, but the conditional probability of collision will be too low for a major distortion of the histograms. It is therefore unlikely that two foci can exist at the same time. The accuracy of an ISI histogram or a death rate plot depends on the number of firings included in the analysis. Regarding a potential selection bias of a particular type of fasciculation, it should be realized that, understandably, only patients with prominent fasciculations were selected for our study. In addition, the cumulative ISI analysis shown in figure 3 is biased in favor of the more frequently firing FPs, and infrequently fasciculating motor units cannot be classified at all. Indeed,

Figure 3 Histograms of pooled interspike intervals for the two types of fasciculation potentials (A and B)



The inset in A shows the histogram at a higher time resolution. The interspike intervals (ISI) from A (C: gray line) and B (C: black line) were separately transformed into an interval death rate in part C. In both cases, the interval death rate was constant from 500 msec onward, as expected for a Poisson process. The difference in time courses for latencies up to 60 msec is enlarged in the inset.

we found that a minority of 23% of the FPs had discharges originating in the spinal motor neuron. However, they accounted for the majority of the firings that were analyzed. Therefore, our study reveals the distinction of two basic mechanisms for FP generation but does not provide an estimate on the relative importance of neuronal and axonal mechanisms on the individual FP discharge level or on the individual patient level.

For any excitable membrane, a new action potential is discharged when the combination of the post-spike potential trajectory and input currents reaches the threshold. Under steady state condi-

tions, the sum of all inputs can be considered as random noise.¹⁸ With this assumption, the interval death rate function resembles the shape of the post-spike membrane potential.¹⁹ Below we compare the ISI death rate plots (figure 3C) with membrane properties that can be measured with threshold tracking techniques.³³

The first few milliseconds after an FP, the generating membrane was refractory. In some FPs, firing probability substantially increased around 5 msec after FP discharge. The latter corresponds to the “supernormal” or “superexcitable” period that follows an action potential in myelinated axons.³⁴ An increased supernormality of motor axons has been found in patients with ALS and is associated with the occurrence of fasciculations.³⁵ More recently, two independent studies have confirmed this finding and have attributed the increased supernormality to a reduction in potassium conductance.^{36,37} In another study, axonal excitability was measured over the nerve and at the motor point. In patients with ALS, the abnormalities were more pronounced distally.³⁸ This supports the link with supernormality and could indicate that axonal-type fasciculations are generated distally. The time course of membrane excitability is thus consistent with an axonal membrane generating the FP.

Another argument for a distal origin of axonal fasciculations is repetition with an ISI compatible with the latency of F waves.⁹ With our surface recording technique, an F wave has the same morphology as the preceding FP, and the ISI reveals the locus of generation. Because the root conduction time to the biceps muscle is 5 to 7 msec,³⁹ F waves from a distal site should produce a peak in the ISI distribution at 10 to 15 msec. In the quadriceps muscle, the F-wave latency should be longer. In contradiction to what would have been expected, we found no peak attributable to F waves in our ISI histograms (figure 3A). Thus, the location of the generator for the axonal type of FPs remains unknown. For this, high-density surface EMG recordings of FPs in a distal muscle may be needed. When the motor nerve is accessible and long enough, collision or nerve blocks may be applied to gain additional localizing information.

Short ISIs (<10 msec) can occur during voluntary contraction and are termed doublet discharges. Doublet discharges have been described in normal subjects, mostly at movement initiation and in fatigue.⁴⁰ Transcranial magnetic stimulation and H reflexes can evoke doublets, confirming a spinal origin in normal subjects.^{41,42} These

doublets are caused by delayed depolarization of the spinal motor neuron. However, delayed depolarization is followed by afterhyperpolarization, such that a longer ISI should follow. In this case, the ISI histogram would consist of a peak below 15 msec and a broader peak around 80 to 100 msec, separated by a region without counts between the peaks. Such a combination of the ISI histograms shown in figure 2, B and E, within one motor unit was not encountered. Other authors have described doublets exclusively in neuromuscular disorders and have localized their origin to the periphery.^{43,44} Doublets tend to occur together with FPs, although not necessarily in the same MU.⁴⁴ Closer examination of the joint ISI plot (figure 2C) shows that a third firing can follow a doublet already after a few milliseconds, confirming the absence of afterhyperpolarization. Therefore, the doublets found here are compatible with an axon that exhibits increased supernormality somewhere along its course.

A long refractory period of 25 msec suggests that the membrane became strongly hyperpolarized after discharging a spike. Such firing behavior cannot be explained by the properties of an axon but is consistent with spike origination in the motor neuron itself. A high proportion of these FPs occurred in ISIs of 70 to 200 msec, which is typical for MUs during voluntary contraction. When MUs are recruited, they fire regularly, i.e., each ISI is preceded and followed by an ISI of similar duration. As can be seen for figure 2F, two discharges with an ISI of approximately 100 msec were often preceded or followed by a much longer ISI. These long irregular intervals distinguish FPs from regularly firing MU action potentials.¹

MU action potentials occur in regular discharges, partly because of the action of persistent inward currents. These dendritic sodium and calcium conductances give rise to plateau potentials that support sustained firing.⁴⁵ The threshold for activation of these currents is close to the recruitment threshold, which means that under normal circumstances, long ISIs can only occur at the onset or offset of contractions. Obviously, the generation of FPs in the spinal motor neuron would require the generation of spikes to be distinct from the recruitment of plateau potentials. This phenomenon has been described recently in cultured motor neurons and in a computer simulation.⁴⁶ These neurons responded to a current step with sustained firing. Blockade of persistent sodium conductance resulted in only a single spike. This would imply that the persistent sodium current is lower in fasciculating motor neurons. In-

triguingly, riluzole has been found to block these persistent sodium currents,⁴⁷ but has at the same time a dose-dependent suppressive effect on fasciculations in patients with ALS.⁴⁸ This could indicate that fasciculations in ALS may not be associated with persistent inward currents, but with abnormal phasic input to the spinal motor neuron. Such abnormal impulses form a hyperexcitable cortex should reach a number of low-threshold motor neurons in the spinal cord. Several authors have taken synchronous, complex, or consecutive FPs as evidence for a cortical involvement in the fasciculations of ALS.^{14,15,49} The number of spikes is large in our data set, but not sufficient to prove or exclude the cortical contribution. Thus, whether the neuronal fasciculations are caused by dysfunction of the spinal motor neuron itself or reflect abnormalities in central input needs to be established.

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